Immunodominant Autoepitopes of Type VII Collagen Are Short, Paired Peptide Sequences Within the Fibronectin Type III Homology Region of the Noncollagenous (NC1) Domain

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Autoantibodies to type VII collagen are associated with the blistering diseases epidermolysis bullosa acquisita and bullous systemic lupus erythematosus. We showed previously that these autoantibodies recognize epitopes within the noncollagenous (NC1) region of type VII collagen. That region is composed of fibronectin type III homology units that may contribute to intermolecular cross-linking and basement membrane adhesion functions of type VII collagen. In this study, we defined the specific amino acid sequences recognized by these autoantibodies. By fusion protein analysis, sera from patients with epidermolysis bullosa acquisita and bullous lupus were found to react with two regions within the fourth (E-1) and eighth (E-2) fibronectin homology repeats, each consisting of approximately 100 amino acids. Affinity purification studies showed E-1 and E-2 to be independent and non–cross-reactive epitope regions. These regions were probed further by enzyme-linked immunosorbent assay analysis of overlapping octapeptide sets derived from the amino acid sequences of E-1 and E-2. The results showed two reactive, closely associated octapeptide sequences within each region, both lying in amphipathic portions of fibronectin type III homology repeats. These studies identify short peptide sequences within the NC1 domain of type VII collagen that are targeted independently by autoantibodies. These sequences may play a direct role in determining the properties of type VII collagen that influence adhesion between this molecule and other basement membrane proteins, and their alteration by antibody binding may be the immunopathogenic event underlying epidermolysis bullosa acquisita and bullous lupus. *Key words: epidermolysis bullosa acquisita/bullous systemic lupus erythematosus/autoimmunity/anchoring fibrils. J Invest Dermatol 104: 231–235, 1995*

Type VII collagen (C7) is an epithelial basement membrane protein composed of three identical alpha chains, each consisting of an amino-terminal 145-kD noncollagenous (NC1) domain and a 145-kD carboxyl-terminal collagenous domain [1,2].

Gammon *et al* [3] cloned cDNA for C7 that encoded the majority of the NC1 domain. Analysis of the deduced amino acid sequence revealed motifs characteristic of other multidomain extracellular matrix (ECM) proteins that contribute to tissue-specific organization of the ECM. In particular, the NC1 domain was found to consist of sequential fibronectin type III (FN3)-like repeats and an area homologous to the A-2 domain of vonWillebrand factor [3–6]. Studies of the FN3 and vonWillebrand A domain homology regions of other matrix proteins have demonstrated the capability of these proteins to bind different types of collagen, and have suggested that they may mediate significant protein-protein interactions [7–10]. These are the same characteristics that have been attributed to the C7 NC1 domain.

Epidermolysis bullosa acquisita (EBA) and bullous eruption of systemic lupus erythematosus (bullous SLE) are autoimmune blistering diseases characterized by circulating and tissue-bound IgG autoantibodies to the epithelial basement membrane. It has been demonstrated that these autoantibodies have specificity for C7 [11,12]. It also has been shown by electron microscopy that EBA and bullous SLE result from epidermal-dermal separation due to lamina-densa–dermal dysadhesion [13,14]. This suggests that these antibodies directed against the principal structural components responsible for maintaining lamina-densa–dermal adhesion may play a role in the pathogenesis of these diseases.

In the current study, we used recombinant fusion proteins and synthesis of sequential, overlapping octapeptides corresponding to the NC1 domain to localize more precisely the immunodominant autoantibody epitopes of C7. By mapping the specific areas of autoantibody interaction with the C7 molecule, we hoped to elucidate a mechanism of immunopathogenesis for EBA and bullous SLE, as well as gain insight into which areas of this molecule are crucial to its functional integrity as a lamina-densa–dermal anchor. Knowledge of specific immunologically reactive peptide sequences also would allow comparison of these areas to protein sequence data bases to search for

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Abbreviations: C7, type VII collagen; FN3, fibronectin type III; IIF, indirect immunofluorescence; NC1, noncollagenous-1.

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possible foreign protein immunogens that could support a theory of molecular mimicry in explaining autoantibody production in patients with EBA and bullous SLE.

MATERIALS AND METHODS

DNA The source of DNA fragments for fusion protein expression was a previously described partial C7 cDNA (CVII-1) [3]. It encodes an estimated 75% of the NC1 domain beginning downstream of the 5′ start site and ending at the beginning of the collagenous domain. It encodes 7 1/2 sequential FN3 repeats of 90 amino acids each, followed by a von Willebrand factor A domain homology region of 190 amino acids [3]. DNA corresponding to different regions of CVII-1 was prepared for cloning by restriction endonuclease digestion and DNA amplification by the polymerase chain reaction.

Fusion Proteins DNA fragments corresponding to the regions of NC1 desired for expression were cloned into pGex-2T (Pharmacia LKB Biotechnology, Piscataway, NJ) per the supplier's protocol. pGex-2T glutathione-S-transferase fusion proteins were expressed in JM109 (DE3) *Escherichia coli* cells after addition of isopropyl-β-D-thiogalactopyranoside to log phase cultures. Recombinant clones were screened by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of bacterial lysates for expression of fusion protein of predicted mass. Glutathione transferase fusion proteins were affinity-purified by absorption on glutathione sepharose (Pharmacia LKB Biotechnology) and assayed by SDS-PAGE.

Enzyme-Linked Immunosorbent Assay (ELISA) Affinity-purified glutathione transferase fusion proteins were diluted in 0.1 M carbonate buffer, pH 9.6, and incubated overnight at 4°C in 96-well polystyrene microtiter plates (Costar, Cambridge, MA). The wells were washed with phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 0.25% bovine serum albumin (PBS-Tween-BSA), and blocked with PBS-Tween-BSA. The wells were incubated with sera diluted in PBS-Tween-BSA, then washed with PBS-Tween-BSA. After washing, antibody binding was detected using anti-human alkaline phosphatase-conjugated anti-IgG secondary antibody (Sigma, St. Louis, MO) and developed with p-nitrophenyl phosphate [15]. OD 405 readings for each well were taken with a microtiter plate reader (Molecular Devices, Menlo Park, CA).

Affinity Purification of Sera Affinity-purified glutathione transferase fusion proteins were diluted in 1% BSA and incubated overnight at 4°C with nitrocellulose paper (Schleicher & Schuell, Keene, NH) in a petri dish. This was washed extensively with PBS, pH 7.4, containing 0.05% Tween 20. Patient sera were diluted in 1% BSA and incubated with the nitrocellulose for 2 h at room temperature, then overnight at 4°C on a rotary shaker. The diluted sera were then decanted and saved as "post-absorbed" sera. The fusion protein-specific antibodies were eluted off the nitrocellulose with 0.2 M glycine, neutralized, and concentrated.

Synthesis of Peptides Peptides were synthesized on polystyrene rods in the configuration of a 96-well microtiter plate by the method of Geysen *et al* [16]. The peptide sequences of the E-1 and E-2 suspected epitope regions were synthesized as octapeptides overlapping by seven amino acids (see **Fig 2**). This synthesis was carried out according to the kit manufacturer's instructions (Cambridge Research Biochemicals, Wilmington, DE). Patient sera were analyzed for binding to these octapeptides using a modified ELISA technique. The synthetic peptides were precoated with 2% BSA-0.05% Tween 20-PBS for 1 h at room temperature. Patient sera were diluted in BSA-Tween-PBS and incubated for 1 h with the polyethylene rods bearing the synthetic peptides. The rods then were washed extensively in 0.01 M PBS, and antibody binding was detected using an anti-human peroxidase-conjugated anti-IgG secondary antibody (Sigma), which was developed with 2,2′-azino-bis-(3-ethylbenzthiazadine-6-sulfonic acid) (Sigma). After 1 h incubation in this substrate, the OD 405 of the incubation wells was measured in a microtiter plate reader (Molecular Devices).

The antibodies bound to the synthetic peptides were removed per the manufacturer's instructions (Cambridge Research Biochemicals), and the polyethylene rod-octapeptide system was reused.

Affinity Purification of Sera Using Synthetic Peptide The decapeptide VPRAQGFLLH was synthesized by Multiple Peptide Systems (San Diego, CA). Using a kit supplied by this company, we anchored this peptide to a solid resin for affinity purification of patient sera. Anti-VPRAQGFLLH–specific antibodies were isolated from patient sera according to the protocol supplied with the kit.

Indirect Immunofluorescence (IIF) Assay Affinity-purified patient sera were assayed by IIF using 1.0 M sodium-chloride–split normal human skin as substrate. This procedure has been described previously [17].

Antisera Sera were obtained from EBA and bullous SLE patients. All sera were shown to contain autoantibodies to the epithelial basement membrane of stratified squamous epithelia by IIF on 1.0 M NaCl-split skin. All sera contained antibodies to the dermal side of split skin and reacted with tissue C7 or proC7 by Western immunoblotting, as described previously [3,18]. Control sera were obtained from normal humans and from bullous-pemphigoid patients who have anti-basement membrane antibodies that target hemidesmosome-associated antigens.

RESULTS

Autoantibodies Directed Against Type VII Collagen Recognize Two Distinct, Non–Cross-Reactive Epitopes Previous data suggested the presence of two epitope regions within the NC1 domain of C7: E-1, composed of 295 amino acids corresponding to the first 884 base pairs of the NC1 cDNA; and E-2, composed of 207 amino acids corresponding to base pairs 1372–1992 of the cDNA **(Fig 1)**. Three EBA sera and one bullous SLE sample were affinity-purified against the immobilized fusion proteins E-1 and E-2 as described. This resulted in purified, epitope-specific anti–E-1 and anti–E-2 antibody populations, as well as post-absorbed fractions of sera devoid of these antigen-specific populations of antibodies. ELISA analysis of these sera revealed the following. Affinity-purified anti–E-1 sera reacted strongly with E-1 fusion protein but negligibly with E-2 fusion protein in three of three EBA sera and one of one bullous SLE serum. Similarly, affinity-purified anti–E-2 sera reacted with E-2 fusion protein but not with E-1 fusion protein for all sera. Post-absorbed serum fractions demonstrated a marked decrease in the degree of their reactivity with the fusion protein used for their purification, but showed minimal or no change in the degree of reactivity with the other epitope protein. These data are summarized in **Table I**. These results suggest the existence of two distinct, non–cross-reactive epitopes (represented by fusion proteins E-1 and E-2) within the NC1 region of C7.

Affinity-purified anti–E-1 and anti–E-2 antibodies were assayed by IIF using NaCl-split human neonatal foreskin as substrate. All four sera demonstrated linear staining along the dermal side of the split for both anti–E-1 and anti–E-2 antibodies, the same pattern that is characteristic of the whole EBA and bullous SLE sera in this assay. As expected, the post-absorbed fractions of these sera revealed a decrease in IIF titer with respect to their parent sera. Three of three EBA sera and one of one bullous SLE serum demonstrated complete extinction of immunofluorescence after removal of both anti–E-1–specific and anti–E-2–specific antibody populations by absorption **(Table II)**. This is evidence that these

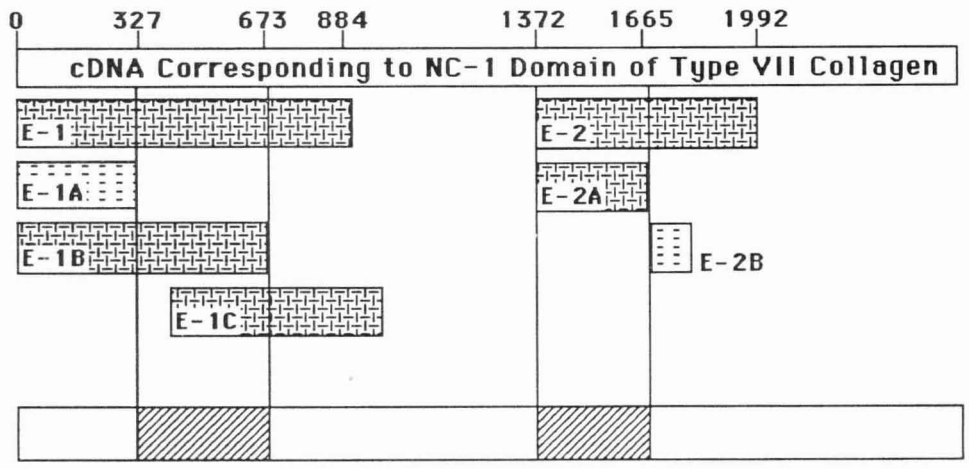

Figure 1. Schematic diagram demonstrating the areas of the NC1 domain represented by our fusion proteins. The cDNA encodes 7.5 FN3 homology repeats starting from the 5′ end and extending to a single region of vonWillebrand factor A domain homology at the 3′ end. The fusion proteins are depicted by bars containing either +++ or −−−, indicating their reactivity or lack of reactivity, respectively, with patient sera in ELISA. E-1 is 295 amino acids (AA) encoded by the first 884 base pairs (BPs) of the cDNA; E-1A, 109 AA, BPs 0–327; E-1B, 224 AA, BPs 0–673; E-1C, 174 AA, BPs 405–927. E-2 is 207 amino acids encoded by base pairs 1372–1992; E-2A, 99 AA, BPs 1372–1665; E-2B, 34 AA, BPs 1664–1765. The *bottom bar* represents the primary structure of the NC1 domain, in which cross-hatching delineates areas of consensus recognition by autoimmune sera.

Table I. ELISA Reactivity of EBA and Bullous SLE Affinity-Purified and Post-Absorbed Sera with E-1 and E-2 Fusion Proteins

Serum[a]	E-1 Fusion Protein	E-2 Fusion Protein
Patient sera		
EBA-1	+	+
EBA-2	+	+
EBA-3	+	+
BSLE-1	+	+
Affinity-purified sera		
EBA-1 anti–E-1	+	−
EBA-2 anti–E-1	+	−
EBA-3 anti–E-1	+	−
BSLE-1 anti–E-1	+	−
EBA-1 anti–E-2	−	+
EBA-2 anti–E-2	−	+
EBA-3 anti–E-2	−	+
BSLE-1 anti–E-2	−	+
Post-absorbed fraction of sera purified against E-1		
EBA-1	−	+
EBA-2	−	+
EBA-3	−	+
BSLE-1	−	+
Post-absorbed fraction of sera purified against E-2		
EBA-1	+	−
EBA-2	+	−
EBA-3	+	−
BSLE-1	+	−

[a] BSLE, bullous SLE.

fusion protein epitopes (E-1 and E-2) correspond to C7 epitopes in native human skin and, at least for one bullous SLE and three EBA sera, account for the full extent of anti-C7 autoantibody binding.

Analysis of E-1 and E-2 Using Small Recombinant Fusion Proteins Within These Domains To localize further epitopes within the relatively large E-1 and E-2 regions, we created smaller fusion proteins representative of areas within these two broad domains. For E-2, we constructed fusion proteins E-2A, corresponding to the first 99 amino acids of this 207-amino acid region; and E-2B, corresponding to amino acids 100–133 **(Fig 1)**. Autoimmune EBA and bullous SLE sera were reacted with these fusion proteins by ELISA. E-2A was recognized by 10 of 12 EBA and seven of seven bullous SLE sera; E-2B was recognized by zero of five EBA and zero of four bullous SLE sera **(Table III)**. Control sera from four normal humans and two patients with bullous pemphigoid were tested in all assays and showed no reactivity with any of the fusion protein fragments. These data suggest that the predominant E-2 epitope lies within the 99-amino acid E-2A region.

Anti–E-2A–specific antibody populations were affinity-purified from one EBA and one bullous SLE serum sample using immobilized fusion protein. ELISA analysis of these anti–E-2A–specific, affinity-purified sera and their post-absorbed fractions revealed the following. The affinity-purified anti–E-2A sera reacted with E-2A and E-2 fusion protein but not with E-1 fusion protein. Post-absorbed fractions of both sera, devoid of anti–E-2A antibodies, showed negligible reactivity with E-2A and E-2. Finally, the affinity-purified anti–E-2A sera stained NaCl-split human skin in an IIF assay with the linear dermal pattern characteristic of anti-C7 antibodies.

Table II. Results of IIF Assays of Autoimmune Sera Before and After Absorption Against E-1 and E-2 Fusion Proteins Using NaCl-Split Human Skin as Substrate

Serum[a]	IIF of Native Serum	IIF of Serum Absorbed Against E-1 and E-2
EBA-1	1:80	0 at 1:5
EBA-2	1:80	0 at 1:5
EBA-3	1:160	0 at 1:5
BSLE-1	1:40	0 at 1:5

[a] BSLE, bullous SLE.

Table III. ELISA Reactivity of EBA and Bullous SLE Sera with Fusion Protein Fragments

Fusion Protein	EBA Sera	BSLE Sera[a]
E-1A	0/7	0/5
E-1B	4/6	2/4
E-1C	4/5	0/4
E-2A	10/12	7/7
E-2B	0/5	0/4

[a] BSLE, bullous SLE.

These data suggest that the E-2 epitope is confined to the E-2A region and that antibodies with this epitope specificity also recognize native C7 in human skin. Thus, we have identified an immunologically significant epitope within the NC1 domain of C7.

A similar evaluation of E-1 was performed by producing fusion proteins corresponding to areas within this epitope: E-1A, corresponding to the first 109 amino acids of E-1; E-1B, containing the first 224 amino acids; and E-1C, containing amino acids 136–309 encoded by the NC1 cDNA **(Fig 1)**. By ELISA, zero of seven EBA and zero of five bullous SLE sera reacted with E-1A; four of six EBA and two of four bullous SLE sera reacted with E-1B **(Table III)**. Control sera from four normal humans and two patients with bullous pemphigoid were tested in all assays and showed no reactivity with any of the fusion protein fragments.

For a single reactive epitope region to produce these results, it would have to lie within the area shared by E-1B and E-1C, but outside the area of E-1A. Thus, we consider this 115-amino acid region to represent the predominant E-1 epitope.

Anti–E-1C–specific antibody populations were isolated by affinity purification of two EBA patient sera. By ELISA, these affinity-purified anti–E-1C sera revealed strong reactivity with both E-1C and E-1 fusion proteins. There was no reactivity of these sera with E-2. Post-absorbed sera from these purifications, devoid of anti–E-1C–specific antibody populations, demonstrated only minimal reactivity with E-1 and E-1C fusion proteins. In an IIF assay, the affinity-purified anti–E-1C sera stained NaCl-split human skin with the linear dermal pattern characteristic of anti-C7 antibodies.

Thus, we have localized the predominant C7 epitopes to two small, distinct areas within the NC1 domain of that molecule: E-1, a 115-amino acid region located along FN3 repeat number 4; and E-2, a 99-amino acid region within FN3 repeat number 8.

Use of Synthetic Peptides to Localize More Precisely the E-1 and E-2 Epitopes Once the epitopes were narrowed to these relatively short segments (E-1, 115 amino acids; E-2, 99 amino acids), we used peptide synthesis to investigate these regions further. Using a commercially available kit, sequential, overlapping octapeptides, derived from the amino acid sequences of these two short reactive segments, were synthesized and anchored to polyethylene rods arranged in the configuration of a 96-well microtiter plate **(Fig 2)**. This method allows immunologic analysis of every possible 8-amino acid stretch within the peptide, enabling precise localization of specific areas of high-affinity antibody binding. These peptide sets were analyzed by ELISA for reactivity with EBA and bullous SLE sera **(Fig 3)**.

For the E-2 epitope, octapeptides covering the 99-amino acid sequence of the E-2A fusion protein were synthesized. By ELISA, four of the five sera tested (two of three EBA and two of two bullous SLE) demonstrated the most vigorous response to the octapeptide NH_2-VPRAQGFL, and the fifth serum reacted most strongly with a partially overlapping peptide, NH_2-LPWEPVPR.

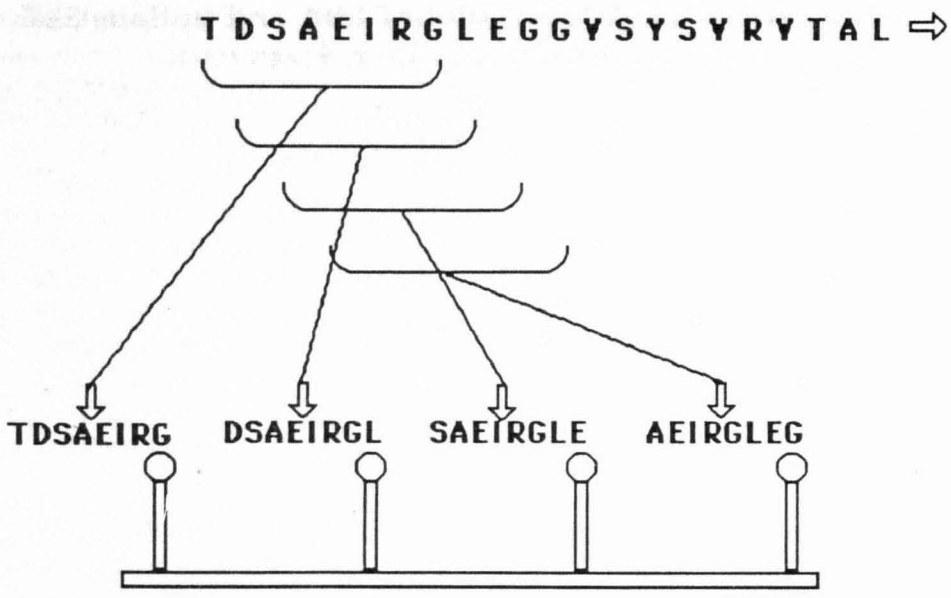

Figure 2. Diagram depicting the method of synthesis of sequential overlapping octapeptides for individual ELISA assay. The amino acid sequence of the E-2A fusion protein is displayed at the top of the figure. *Brackets* demonstrate how octapeptide segments of that sequence are synthesized and anchored to polyethylene rods. Each rod bears a unique peptide that can be assayed by ELISA, allowing determination of serum reactivity with every possible eight-amino acid stretch within the sequence.

Lesser degrees of recognition were evident for the same four of five against a second region of sequential, overlapping peptides—NH_2-LGTLHVVQ, NH_2-GTLHVVQR, and NH_2-TLHVVQRG—which are slightly upstream of the primary region of antibody recognition. Control sera from five normal humans and one bullous-pemphigoid patient demonstrated varying degrees of nonspecific background activity against this E-2A set of octapeptides, but no pattern of specific sequence recognition. Affinity-purified anti–E-2A sera from one EBA and one bullous SLE patient strongly recognized these same two regions. From this, we conclude that the octapeptide NH_2-VPRAQGFL represents the primary antigenic epitope of E-2A, with the upstream sequence of NH_2-LGTLHV-VQRG contributing to a lesser epitope **(Fig 4)**. Thus, we have defined the E-2 epitope to the level of amino acid specificity.

For the E-1 epitope, octapeptides were synthesized representing the first 100 amino acids of the 115-amino acid segment shown to contain the reactive epitope. By ELISA, five of six EBA and two of two bullous SLE sera reacted with the same two areas within this region **(Fig 3)**. The one EBA serum not demonstrating this pattern also reacted poorly with the E-1 fusion protein fragment, and by IIF is known to have a low titer of anti-BMZ antibody. The octapeptide NH_2-RVSWPVP and its overlapping neighbors appear to represent the predominant epitope, and the slightly downstream octapeptide NH_2-IIWRSTQG and its overlapping neighbors represent a second epitope of less avidity **(Fig 4)**. Affinity-purified anti–E-1 sera from one EBA and one bullous SLE patient reacted with this same specific pattern. Control sera from five normal humans and one bullous-pemphigoid patient demonstrated varying degrees of nonspecific background activity against this E-1 set of octapeptides, but no pattern of specific sequence recognition.

The predominant E-2 epitope octapeptide, NH_2-VPRAQGFL, was synthesized in milligram quantities and attached to a solid resin for affinity purification of autoimmune patient sera. Anti-VPRAQGFL sequence-specific antibodies were affinity-purified from two EBA and one bullous SLE sera and tested in an IIF assay against salt-split human skin. All sera stained the dermal side of the split skin with the linear pattern characteristic of the native sera. One normal human serum sample and one from a patient with bullous pemphigoid were purified with the NH_2-VPRAQGFL affinity-purification column as controls. Neither of these controls stained salt-split skin in IIF assays. This result demonstrates that the defined epitope sequence for E-2 represents the native epitope in human skin.

DISCUSSION

In the present study, we have demonstrated the presence of two distinct, non–cross-reactive autoepitopes within the NC1 domain of C7. We have localized these epitopes to paired, short peptide sequences lying within the FN3 repeat homology region of the NC1 domain. Using epitope-specific, affinity-purified EBA and bullous SLE patient sera in IIF assays on human skin substrate, we have shown that the epitopes we have characterized represent the immunodominant epitopes of C7 in native human skin.

Immunologic investigation of C7 has focused on the 145-kD amino terminal NC1 domain of the molecule, as early data demonstrated that patient autoantibodies recognized this region in proteolytically cleaved C7 [11]. In addition, the putative role of the NC1 domain in mediating the protein-protein interactions responsible for the adherence of anchoring fibrils to other ECM components made this area attractive to investigations of blistering-disease pathogenesis. Using fusion protein fragments representing most of the NC1 domain, we demonstrated previously that the autoantibodies from EBA and bullous SLE patients bind to the FN3 homology region of the NC1 domain [19]. These same studies showed no reactivity of patient autoantibodies with a fusion protein corresponding to the vonWillebrand factor A domain homology region. In the current study, we focused on further exploring antibody reactivity with the FN3 homology region.

Elucidation of the specific peptide sequences of the E-1 and E-2 epitopes allowed us to localize them precisely within the NC1

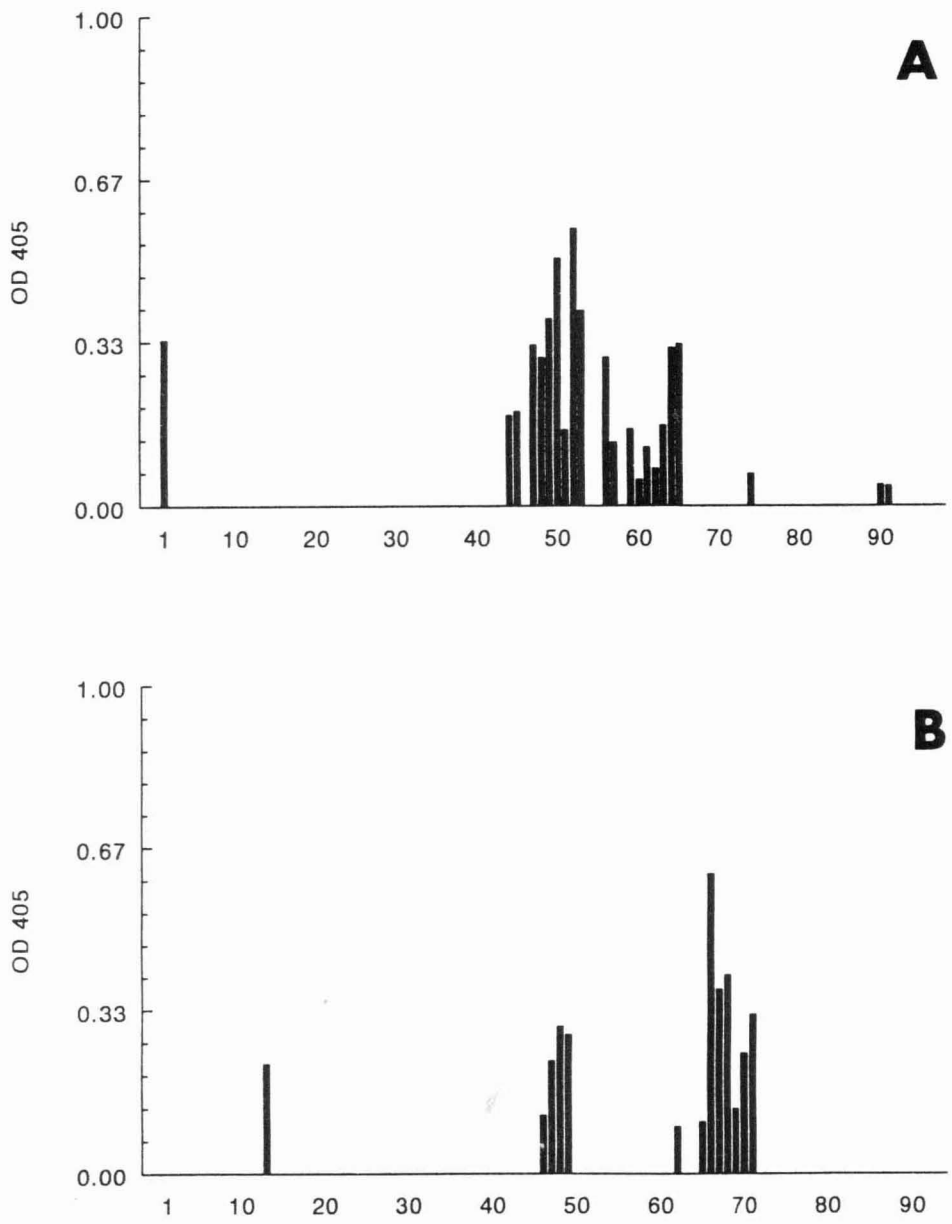

Figure 3. ELISA reactivity of EBA serum with the synthetic octapeptides representing the E-1 and E-2A fusion proteins. The *numbers* along the abscissa indicate individual octapeptides (1 = amino acids [AAs] 1–8 of the sequence; 2 = AA 2–9; 3 = AA 3–10; etc.), and the *bars* depict the degree of reactivity (as measured by OD 405) of that octapeptide with the patient serum. For both E-1 (*A*) and E-2 (*B*), there are two distinct areas of reactivity with most autoimmune sera tested. These are shown in these representative ELISAs.

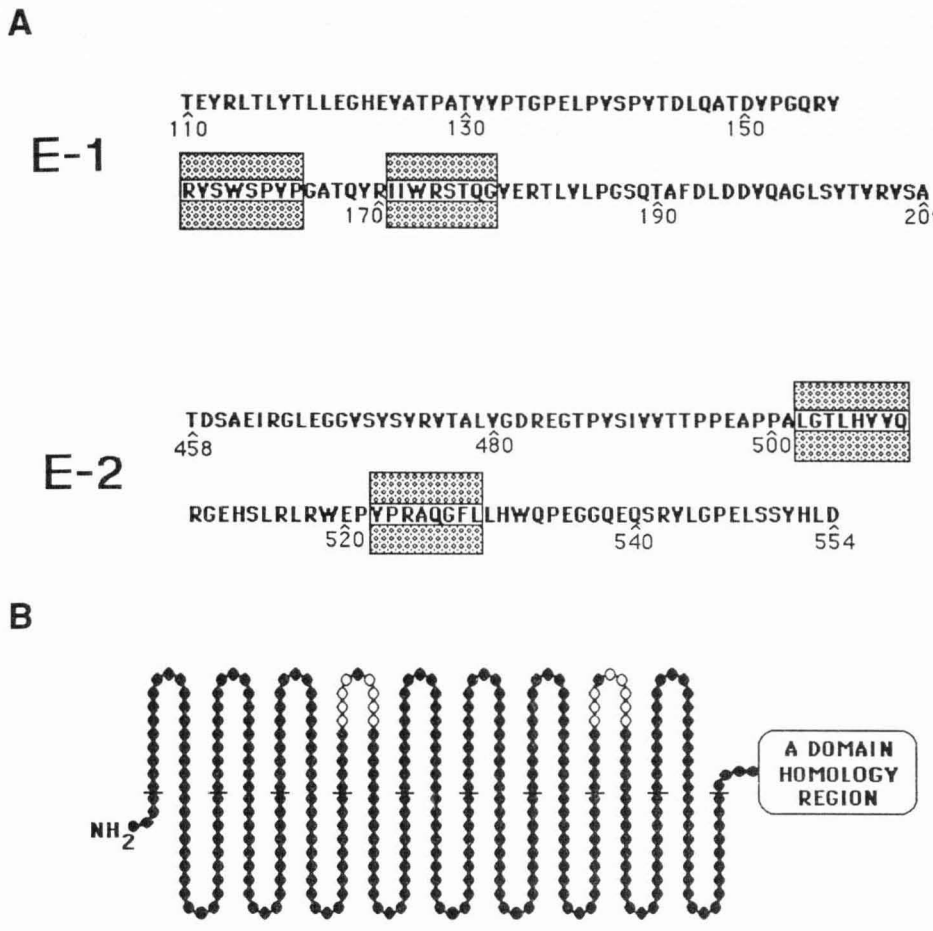

Figure 4. Localization of immunoreactive peptide sequences within epitopes E-1 and E-2, and their location within the FN3 repeats of the NC1 domain. *A*) The amino acid sequences of the E-1 and E-2 regions synthesized as overlapping octapeptides for ELISA testing of autoimmune sera. *Boxed areas* depict the octapeptides recognized most strongly by the sera tested. There are two neighboring reactive sequences for both E-1 and E-2. *B*) Localization of these sequences within the FN3 repeat homology region of the NC1 domain: E-1 along repeat no. 4, and E-2 along repeat no. 8. Alignment is based on invariant tryptophan and tyrosine residues using the GCG software package. Note their location along the turn of the B-pleated sheet of the FN3 repeats. Using the method of local hydrophilicity calculation of Hopp and Woods [20], these epitope regions were both shown to be very hydrophobic.

domain. The two short peptide epitopes of E-1 were found to reside in FN3 repeat number 4 in a hydrophobic region along a turn of the B-pleated sheet structure of this repeat. It is interesting that the two short peptide epitopes of E-2 were similarly shown to reside within an FN3 repeat (no. 8) in a hydrophobic region along the turn of the B-pleated sheet **(Fig 4)**. This unique paired location of these epitopes indicates that these areas of the C7 molecule may be structurally vital to the functional integrity of C7 as a matrix component. One can easily imagine that antibody binding along these B-pleated sheet turns could disrupt the secondary structure of the FN3 repeat area, which in turn could lead to altered matrix-binding properties of this region or an inability of such distorted C7 molecules to aggregate appropriately into anchoring fibrils. Either of these mechanisms could lead to lamina-densa–dermal dysadhesion through impairment of anchoring-fibril function and result in an immunopathogenic event that would explain the blistering of EBA and bullous SLE. These epitopes also may represent areas of molecular abnormality in patients with inherited forms of dystrophic epidermolysis bullosa, in which structural alterations are mediated not through antibody binding, but by improper protein synthesis due to errors in DNA coding for these regions. These studies add to the mounting evidence that the NC1 domain of C7 and, in particular, the region of FN3 homology is significant in mediating the interaction between anchoring fibrils and other matrix proteins.

Knowledge of these specific autoepitope sequences also allowed us to compare them to a protein sequence data bank to search for molecular homology that could substantiate a claim for molecular mimicry as a mechanism of immunogenicity in EBA and bullous SLE. A homology search of the Swissprot data base revealed no significant homology between the main autoreactive sequences and any sequences in that data base.

Because of the limitations of the experimental techniques employed, our investigation was confined to identifying the major areas of antigenicity within the NC1 domain of C7. More specific serum absorption studies using synthetic peptides to isolate antigen-specific antibody populations for each epitope sequence would allow more precise definition of C7 antigenicity. Such antigen-specific absorption studies are necessary to confirm our suspicion that the epitopes we have identified do indeed correspond to the full complement of autoantibodies to native C7. These investigations should be pursued as a part of future projects attempting to clarify C7's role as an autoantigen.

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